

Appendix H:

2016 Eelgrass Monitoring Framework



Technical Memorandum

Reference: 016018.500
Date: June 13, 2016
To: Billy Plauché, Plauché & Carr LLP
From: Greg O'Connell, MS with assistance from Tamre Cardoso, Ph.D.
Subject: Framework for Coast Seafood's 2016 Eelgrass Monitoring Plan

Background

This document summarizes the proposed framework of the 2016 eelgrass (*Zostera marina* L.) monitoring plan and sampling design prepared by SHN Engineers & Geologists (SHN) and TerraStat Consulting Group (TerraStat). This framework will allow the evaluation of the effects of Coast Seafoods Company's revised Humboldt Bay Shellfish Aquaculture Permit Renewal and Expansion project (Project) (Figure 1) on eelgrass areal extent and shoot density, in accordance with the *California Eelgrass Mitigation Policy and Implementing Guidelines* (CEMP) (NOAA, 2014). The project entails a phased implementation approach where approximately one third of the proposed new shellfish cultivation will occur as Phase 1 and the remainder of the expansion (Phase 2) will occur after monitoring of Phase 1 is completed. The goal of the eelgrass monitoring plan is to quantify effects the four proposed shellfish cultivation scenarios have on eelgrass shoot density and areal extent. Eelgrass monitoring data collected during Phase 1 will be used to determine if Phase 1 and proposed mitigation yield an overall decrease, increase, or neutral change in eelgrass.

Monitoring Plan

The four cultivation scenarios listed below as part of Phases 1 and 2 will be monitored as part of this eelgrass monitoring plan. Monitoring of the cultivation method proposed in Phase 2 will be added to the three treatments associated with Phase 1 in order to evaluate the assumption that Phase 2 will have a neutral effect on eelgrass.

Phase 1 expansion: approximately 210 acres of new culture utilizing the following cultivation methods:

- Basket-on-line, where line spacing alternates between 9 feet and 16 feet between lines
- Cultch-on-line, where lines are double hung and consistently spaced 10 feet apart

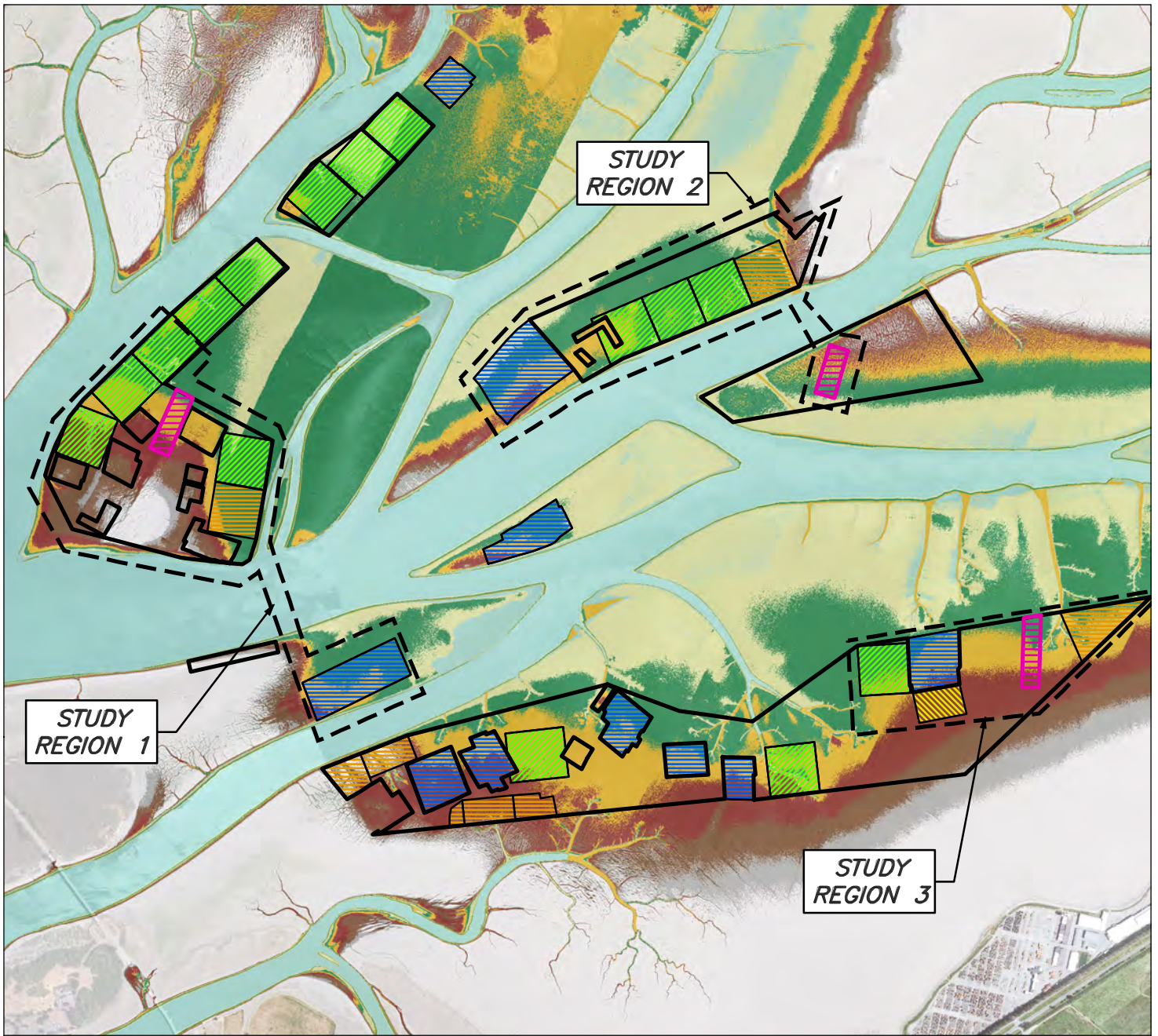
Phase 1 mitigation: Conversion of 100 acres of existing culture to lower line density

- Conversion of existing culture from single-hung 2.5-foot spacing between lines to 10 feet between lines that are double-hung.

Phase 2 expansion: additional 412 acres of new culture

- Cultch-on-line, where lines are single hung and consistently spaced 10 feet apart

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EXPLANATION

REFERENCE SITES

STUDY REGIONS

PHASE I AND II COMBINED

PHASE 1

BASKET-ON-LINE

CULTCH-ON-LINE: DOUBLE-HUNG

CULTCH-ON-LINE: SINGLE-HUNG

MITIGATION AREAS

ELEVATION RANGES (FEET)

-57.88 TO -1

-1 TO -0.5

-0.5 TO 0

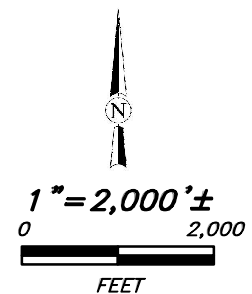
0 TO 0.5

0.5 TO 1

1 TO 1.5

1.5 TO 2

2+



DATA SOURCES: IMAGE (NOAA, 2009); ELEVATION (PWA, 2014); PHASE I & II COMBINED, PHASE 1, AND MITIGATION AREAS (CONFLUENCE, 2016)

Changes in eelgrass areal extent over time within the project area (in addition to reference sites) will be assessed by annual comparisons of aerial imagery. Changes in eelgrass shoot density over time will be assessed through annual ground-based monitoring of the same randomized locations for each treatment. Based on CEMP guidelines, monitoring is proposed to occur for three years; one year prior to project implantation and two years after project implementation. Ground-based field work is limited to tides -1-foot mean lower low water (MLLW) or lower that occur May through September. Aerial imagery collection will be limited to occasions where these negative tides coincide with a sun angle of 30 degrees or higher.

Sampling Design

A stratified, two-stage cluster sampling design has been developed to balance realistic field implementation and spatial coverage. The monitoring area is divided into five strata that consist of the following treatment groups: 1) mitigation (convert existing), 2) new cultch-on-line double-hung 10-foot spacing, 3) new cultch-on-line single-hung 10-foot spacing, 4) new basket-online with alternating 9- and 16-foot spacing between lines, and 5) reference sites.

Within each stratum, 100- by 100-foot primary sampling units (plots) will be randomly selected without replacement from all possible 100- by 100-foot plots within the stratum. Within each primary sampling unit, three culture lines (secondary sampling units) will be randomly selected without replacement from among the total secondary sampling units for mitigation, cultch, or basket culture. Multiple quadrats will be sampled at four random locations along each line with placement to the left or right side of a line also randomized. The total number of quadrats placed may vary by treatment group.

All plots, lines, and quadrat locations will be surveyed for baseline conditions (prior to the implementation of mitigation or Phase 1 of the expansion project). These same locations will be re-surveyed for two consecutive years after implementation of mitigation and Phase 1 of the expansion project.

Stage 1: Aquaculture Treatment Study Sites

In order to allow more survey time, the full study area has been divided into three pre-selected regions that are representative of the entire area. The nine plots for each treatment and reference site (that is, the five strata discussed above) will be randomly chosen from these three pre-selected regions¹ within the project area (Figure 1). For each treatment or reference site within a region, low (-0.5 to 0 feet), mid (0 to 0.5 feet), and high (0.5 to 1.0 feet MLLW) elevations² is sampled within a 100- by 100-foot plot. To avoid unnecessary increases in eelgrass shoot density variance at the plot level, plots will avoid elevation transition zones and areas with sparse or no eelgrass interpreted from aerial imagery.

1 Focusing on pre-selected regions minimizes travel time (allowing more survey time) from otherwise more randomly distributed study plots across the bay.

2 The -0.5 to +1-foot elevation ranges comprise 80 percent of the proposed expansion project and 95 percent of the proposed mitigation areas.

Stage 2: Line Sampling Methodology

Stage two of the monitoring plan sampling design quantifies changes in eelgrass shoot density that results from increased or decreased shellfish cultivation activity on a per-line basis. This is accomplished by randomly selecting three 100-foot cultivation lines that contain eelgrass within Stage 1 plots, then sampling eelgrass shoot density at four random positions along each of the three 100-foot cultivation lines (Figures 2, 3, and 4). During initial randomization of the sampling design, lines that do not contain eelgrass will not be surveyed; the next randomly chosen line number will replace that line. At four random positions on the 100-foot line, a series of quadrats running perpendicular to the cultivation line will be placed to quantify changes in eelgrass shoot density on a gradient going away from the line, where odd numbers along the 100-foot line are sampled to the left of the cultivation line and even numbers are sampled to the right. Each series of quadrats extend to a location representative of a mid-point between lines. The following metrics will be recorded at each quadrat location: eelgrass percent cover, eelgrass shoot density, presence/absence of eelgrass flowering shoots, macrophyte seaweed percent cover, and substrate composition.

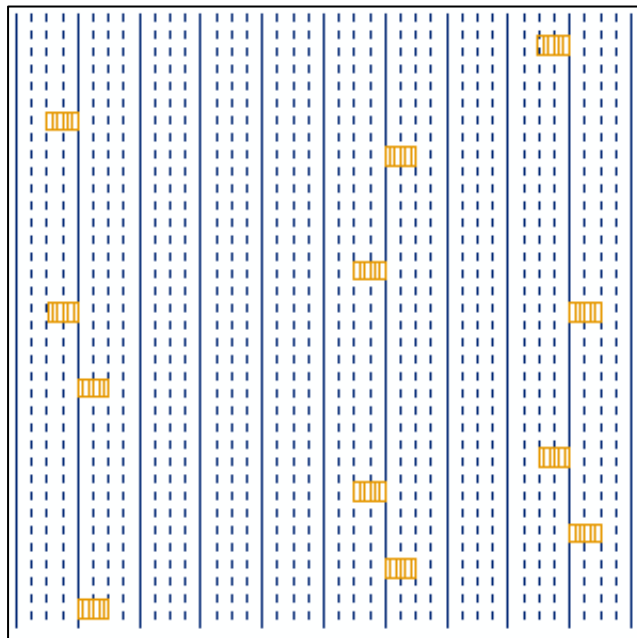


Figure 2. Example of Stage 2 sampling arrangement for mitigation areas where a subsample of three 100-foot (ft) lines (solid lines spaced 10 ft apart; dashed lines represent removed longlines) are each surveyed with a series of six 0.83- by 1.5-ft (1.25 square foot [ft²] [0.116 square meter [m²]]) quadrats extending from under to 5 ft from the line at four locations.

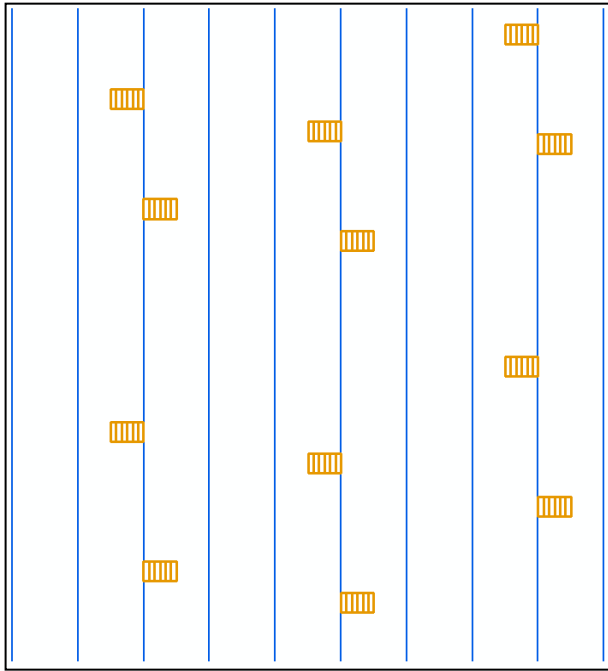
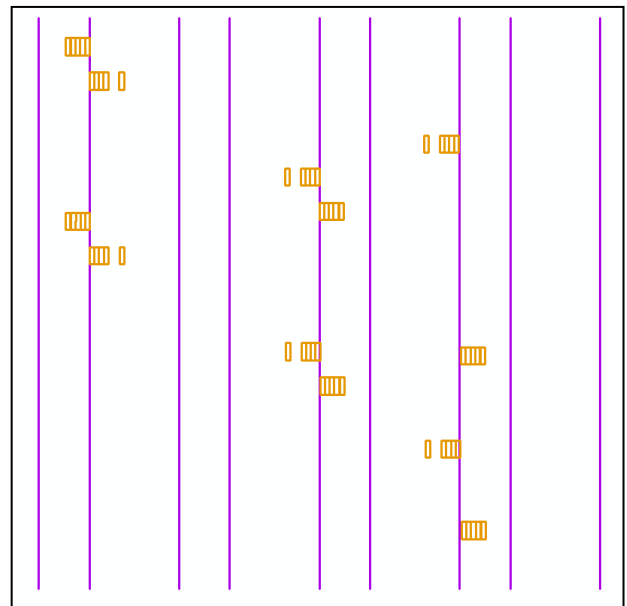


Figure 3. Example of Stage 2 sampling arrangement (for both single and double hung) cultch-on-line spaced 10 ft apart. A subsample of 100-ft lines (blue lines spaced 10 ft apart) are each surveyed with a series of six 0.83- by 1.5-ft (1.25 ft² [0.116 m²]) quadrats extending from under to 5 ft from the line at four locations.

Figure 4. Example of Stage 2 sampling arrangement for basket-on-line with two lines spaced 9 feet apart and 16-ft gaps between paired lines. Each line in a subsample of 100-ft lines is surveyed with a series of five 0.83- by 1.5-ft (1.25 ft² [0.116 m²]) quadrats extending from under to the midpoint between lines at four locations.



Power Analysis and Sample Size Selection

In order to estimate the appropriate number of 100- x 100-foot plots that need to be sampled, we characterized system variability. Two sources of data were available for characterizing background variability: 1) 2015 baseline sampling within the study area, and 2) 2007 – 2010 SeagrassNet data from North Bay. The 2015 data set included shoot density counts on 90, 100- x 100-foot plots (three transects per plot and six to eight 0.0625-m² quadrats per transect). The 2007 – 2010 SeagrassNet data set had July shoot density data for three transects over three years and two transects over four

years. Each transect was subsampled with 12, 0.0625-m² quadrats. These two data sets were used to estimate mean shoot densities and intraannual variability. The SeagrassNet data set was also used to determine interannual variability at the quadrat level for consecutive survey years.

Eelgrass shoot densities are highly variable. Shoot densities at the plot level in the 2015 data set ranged from 7.33 to 126.22 shoots per m² with standard deviations ranging from 13.597 to 61.819 shoots per m². Table 1 shows some paired-difference summaries for each two-year period based on the SeagrassNet data set.

Table 1. Means, Standard Deviations, and Correlations for Eelgrass Shoot Density from Baseline Surveys

Data Set	Mean Paired-Differences in Quadrat Shoots per m ²	Standard Dev. of Paired-Differences in Quadrat Shoots per m ²	Observed Correlations
2007 - 2008	Ranged from -30.67 to 10.7	Ranged from 49.4 to 73.1	Varied between -0.23 to 0.53
2008 - 2009	Ranged from 2.7 to 38.7	Ranged from 25.4 to 71.7	Varied between -0.39 to 0.78
2009 - 2010	Ranged from -16 to -12	Ranged from 28.9 to 37.4	Varied between 0.44 to 0.73

Sample sizes and the power to detect an increase in the mean difference per primary unit based on matched-paired differences among observed transects were evaluated through simulation. Simulation methods allow for the incorporation of correlations between years. Data were simulated using observed means and standard deviations from randomly sampled 2015 data, assuming fixed correlation of 0.6. Power was evaluated for fixed annual increases or decreases from 5 to 25% over a range of from 6 to 12 primary plots per stratum. The estimated power to correctly reject a null hypothesis of no difference, in favor of an alternative of an increase in the population mean difference in eelgrass shoot density at a 5% significance level is shown in Table 2. Results based on a 10% significance level are shown in Table 3. The results are also shown graphically in Figures 5 and 6. All estimates are based on 1,000 simulations. There is a fairly clear jump in power from $n = 6$ to $n = 9$ and less so between $n = 9$ and $n = 10$. For fixed correlation and fixed increases, increases in power do not appear as large when going from $n = 10$ to $n = 11$, or $n = 12$. Power estimates for testing alternative hypotheses for decreases in the population mean difference in eelgrass shoot density are shown in Tables 3 and 4, and Figures 7 and 8; estimated power is similar to that observed for increases.

Although the correlation was set to 0.6, the correlation for any particular simulation varied. Over 1000 simulations, correlations varied from about 0.46 to 0.93, with a mean correlation of about 0.75. This does not include any negative correlations as observed in the SeagrassNet data. Further, the average of 0.75 is higher than most of the observed interannual correlations. If the interannual correlation between quadrats is less than 0.75, the power to detect changes in eelgrass shoot density will likely be lower for fixed levels of change.

Table 2. Estimated power for one-tailed paired-difference *t*-tests ($\alpha = 0.05$) by effect size and plot sample size for fixed increases, based on 1000 simulations using correlation of 0.6. This is equivalent to a two-tailed test at $\alpha = 0.10$.

Effect Size	<i>n</i> = 6	<i>n</i> = 9	<i>n</i> = 10	<i>n</i> = 11	<i>n</i> = 12
5%	0.17	0.23	0.27	0.28	0.30
10%	0.40	0.59	0.66	0.69	0.70
15%	0.67	0.84	0.89	0.93	0.93
20%	0.82	0.96	0.98	0.99	0.99
25%	0.91	0.99	1	1	1

Table 3. Estimated power for one-tailed paired-difference *t*-tests ($\alpha = 0.10$) by effect size and plot sample size for fixed increases, based on 1000 simulations using correlation of 0.6. This is equivalent to a two-tailed test at $\alpha = 0.20$.

Effect Size	<i>n</i> = 6	<i>n</i> = 9	<i>n</i> = 10	<i>n</i> = 11	<i>n</i> = 12
5%	0.32	0.40	0.42	0.44	0.47
10%	0.62	0.75	0.78	0.82	0.83
15%	0.83	0.93	0.96	0.97	0.98
20%	0.93	0.99	0.99	0.99	1
25%	0.97	1	1	1	1

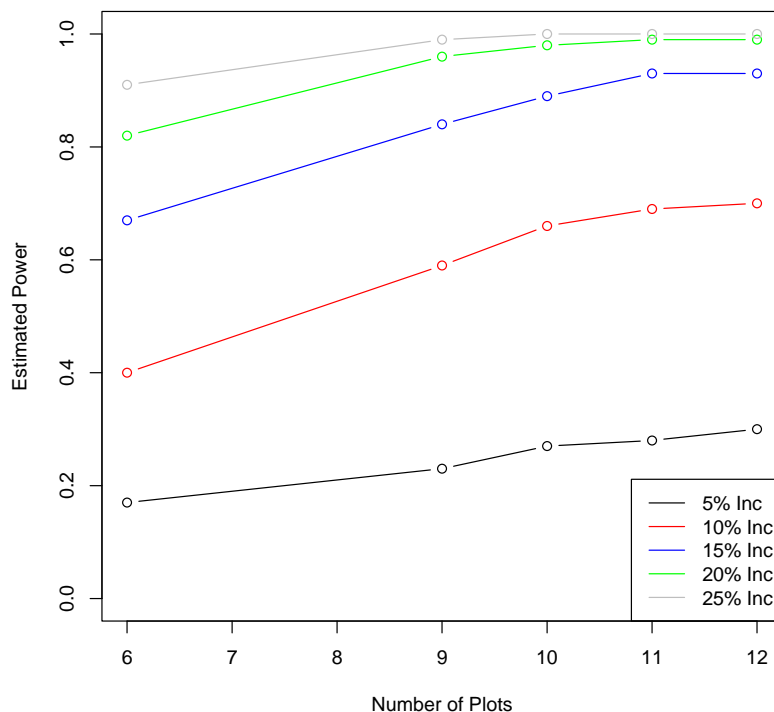


Figure 5. Estimated power for fixed numbers of primary plots and given percent increase based on a one-tailed 5% significance level. Simulated data had an average correlation of 0.75. Given the high variability of the baseline data, simulated data generally covered the range of observed interannual correlations.

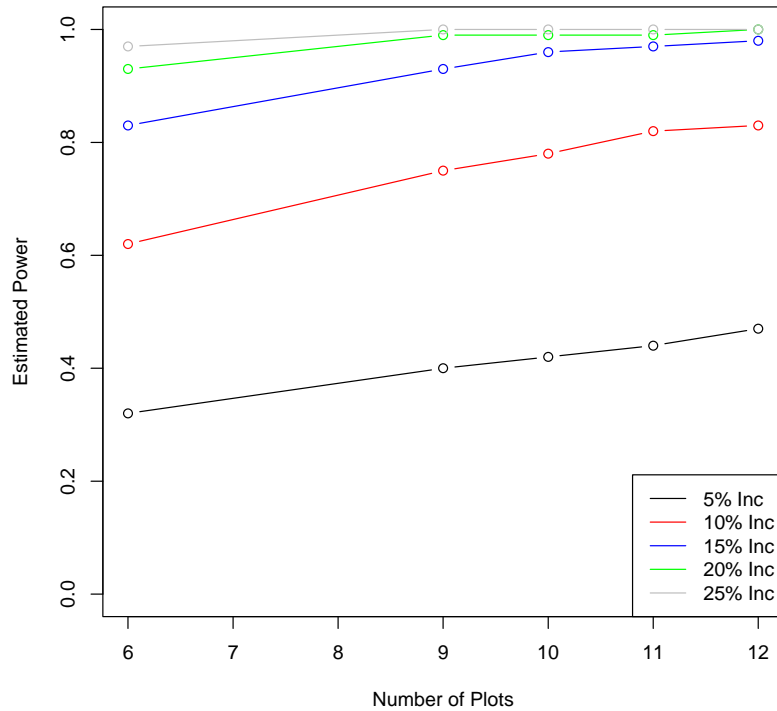


Figure 6. Estimated power for fixed numbers of primary plots and given percent increase based on a one-tailed 10% significance level. Simulated data had an average correlation of 0.75. Given the high variability of the baseline data, simulated data generally covered the range of observed interannual correlations.

Table 4. Estimated power for one-tailed paired-difference *t*-tests ($\alpha = 0.05$) by effect size and plot sample size for fixed decreases, based on 1000 simulations using correlation of 0.6. This is equivalent to a two-tailed test at $\alpha = 0.10$.

Effect Size	<i>n</i> = 6	<i>n</i> = 9	<i>n</i> = 10	<i>n</i> = 11	<i>n</i> = 12
5%	0.17	0.24	0.28	0.30	0.33
10%	0.41	0.61	0.65	0.68	0.74
15%	0.68	0.84	0.89	0.91	0.94
20%	0.83	0.97	0.97	0.99	0.99
25%	0.91	0.99	1	1	1

Table 5. Estimated power for one-tailed paired-difference *t*-tests ($\alpha = 0.10$) by effect size and plot sample size for fixed decreases, based on 1000 simulations using correlation of 0.6. This is equivalent to a two-tailed test at $\alpha = 0.20$.

Effect Size	<i>n</i> = 6	<i>n</i> = 9	<i>n</i> = 10	<i>n</i> = 11	<i>n</i> = 12
5%	0.29	0.37	0.43	0.44	0.50
10%	0.61	0.77	0.80	0.81	0.86
15%	0.83	0.93	0.96	0.96	0.97
20%	0.93	0.99	0.99	1	1
25%	0.98	1	1	1	1

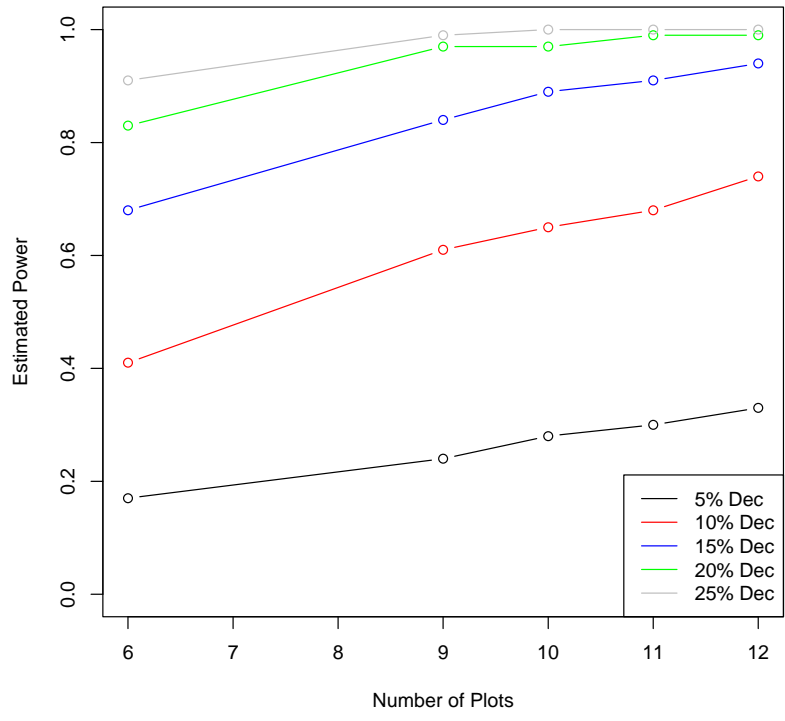


Figure 7. Estimated power for fixed numbers of primary plots and given percent decrease based on a one-tailed 5% significance level. Simulated data had an average correlation of 0.75. Given the high variability of the baseline data, simulated data generally covered the range of observed interannual correlations.

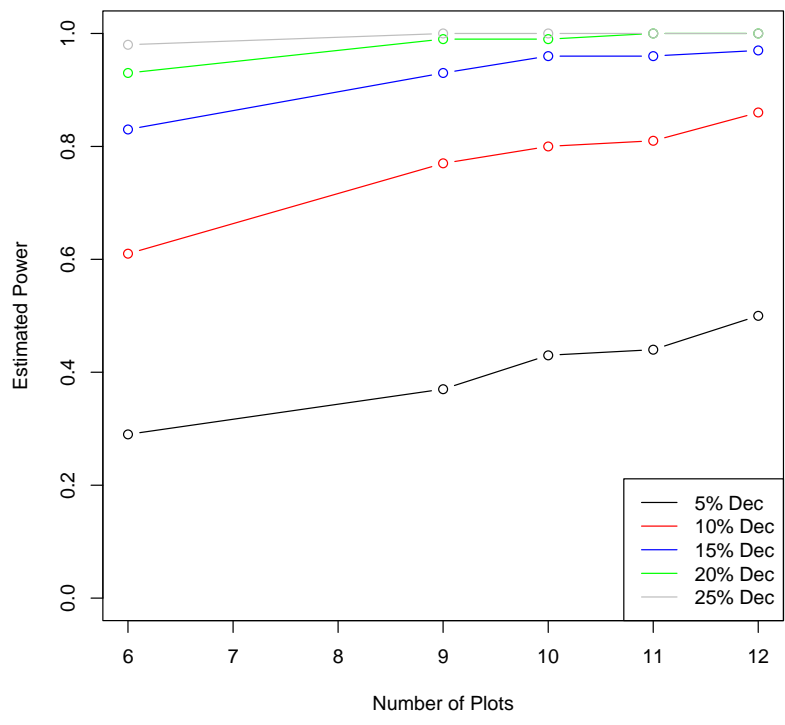


Figure 8. Estimated power for fixed numbers of primary plots and given percent decrease based on a one-tailed 10% significance level. Simulated data had an average correlation of 0.75. Given the high variability of the baseline data, simulated data generally covered the range of observed interannual correlations.

Assumptions and Caveats for Simulations

The assumptions or caveats listed below pertain to creation of sample data sets for the simulations.

- The 2015 baseline data set is comprised of 30, 100- x 300-foot blocks (labeled blocks 0 – 29), with each block split into 3, 100- x 100-foot plots for a total of 90, 100- x 100-foot plots. Blocks 0, 2, and 3 were omitted due to high numbers of zeros, yielding a data set with 27 blocks and 81, 100- x 100-foot plots.
- Data sets were created by randomly sampling $n = 6, 9 - 12$ plots without replacement from the 81 available plots. These plots were the primary sampling units.
- The 2015 baseline data set has samples from plots across a range of elevations from -0.75 to 0.25 feet MLLW. Elevations above 0.25 feet MLLW were omitted by exclusion of Block 0. The data used in these simulations does not cover the full range of elevations that we may encounter in the study area.
- The 2015 baseline data set included three random transects per plot, each sampled using size 0.0625 m^2 quadrats. The simulations used all three transects within the selected plots. We did not investigate the effect of the number of transects on power, because we expect counts along transects within a plot to be more similar than counts along transects in other plots. We also used the six sampled quadrats along each transect, which yielded 0.375 m^2 of total sampling area per transect. This is less area than our proposed sampling under this plan.
- Simulations were based on quadrat counts that were scaled to the number of shoots/ m^2 .
- In order to compare possible differences based on the number of sampled plots, we looked at a set of effect sizes that ranged from 5% to 25% increases in increments of 5%.
- Effect sizes were added to the data after generating bivariate random standard normal variables with a fixed correlation of 0.6. In order to scale up according to the estimators for a two-stage cluster sample, all densities were converted to an expected number of shoots per transect, with further scaling to an expected number of shoots per plot. Scaling took into account the size of the sampled quadrats. Note that shoot densities do not appear to be normally distributed; rather, the distributions appear to be unimodal and right-skewed.
- We assumed that all 100- x 100-foot plots will contain $M_i = 10$ transects (or $M_i = 8$ transects for basket sites) and that $m_i = 3$ transects per plot will be sampled for the mitigation sites.
- The estimators for two-stage cluster sampling applies a finite population correction factor based on N , the total number plots in the sampling frame. Because the data set used for simulation has 81 plots, N was taken to be 81.
- The power analysis is based on one-tailed, matched-pairs t -tests looking at increases or decreases. Data were matched at the quadrat level. Further, the variance estimators used for the hypothesis tests were based on estimators for two-stage cluster sampling (equations provided in the Appendix at the end of this memo).

It should be noted that *t*-tests require that the underlying distribution of the populations is normal. For the baseline data on hand, the distribution of shoot density at the quadrat level is not normally distributed. The data are right-skewed with one or two modes, and with several high outliers. Non-parametric *t*-test alternatives or permutation tests may be more appropriate means of processing the monitoring data.

Monitoring Data Analysis

The power analyses assume a hypothesis testing framework for analyzing the monitoring data. For a matched pairs analysis approach, this framework may be used to test for evidence of increases or decreases in eelgrass shoot density between any pairs of years. This will result in an analysis of before (baseline) versus after (treatment). The current monitoring, however, is not set up as a true before-after impact control study (BACI). Without the collection of a second baseline year of data from the mitigation lift sites, a BACI approach for the mitigation lift stratum will not be possible, because we will not have control data for two time points. A BACI analysis approach may be possible for the other treatment strata by using two years of reference site data as control data. This assumes that the shoot densities observed in the reference sites is representative of the shoot densities that would be observed before treatment at the Phase 1 expansion sites.

Other analysis approaches may be used with these data. This includes construction of confidence intervals for mean shoot densities in a given year or confidence intervals for mean differences between paired years. Additionally, statistical modeling methods (such as, the use of generalized linear mixed models) may provide additional insights into the variance components of eelgrass shoot density among years and treatments.

Additional Analyses Proposed for Monitoring Plan

High resolution areal imagery (3-inch typical pixel size) was obtained from Cessna airplane flights flown at 3,000-foot elevation on May 11 and June 8, 2016, for the project area. Very high resolution imagery (1-centimeter typical pixel size) was obtained from an unmanned aerial vehicle flown at 50 feet elevation within three 8-acre locations in the western, central, and eastern portions of the project area; each location covered a swath perpendicular to the elevation gradient. Photogrammetry technicians are currently processing those data. These and future aerial imagery data will be used to assess changes in eelgrass areal extent over time.

Conclusion

This draft monitoring and sampling plan was designed to provide a framework for assessing changes in eelgrass areal extent and shoot density after implementation of shellfish aquaculture in areas of Humboldt Bay, while keeping in mind practical sampling constraints. A specific focus of this plan was to monitor eelgrass shoot density for assessment of impacts due to shellfish aquaculture. The design was crafted to allow for estimation across various strata within the study area. Making use of matched-pairs is natural given this temporal design. Provided that assumptions are met, *t*-tests are one of the most common and easily implemented analysis methods for assessing significant changes in means pre- and post-impact. The purpose of the simulations was to estimate statistical power that can be expected for given sample sizes when testing for increases or decreases in shoot density, in light of the natural variability of the system. Based on the simulations, a sample size of $n = 9$ primary plots per stratum should provide good power to detect increases or decreases of 15% and high power to detect increases or decreases of 20% or more (one-tailed tests with $\alpha = 0.10$). One-tailed tests were used, because we expect likely increases in eelgrass in mitigation lift plots. For Phase 1 expansion plots, one-tailed or two-tailed tests may be appropriate depending on whether or not a decrease in eelgrass is expected. Increasing the sample size up to $n = 12$ does not provide a substantial increase in power. Assuming variability similar to that observed in the 2015 and SeagrassNet data, estimated power will decline if the interannual correlation between quadrats declines.

Appendix: Estimators for Two-Stage Cluster Sampling

A number of quantities within each stratum may be estimated based on two-stage cluster sampling, as defined below.

Given the following definitions,

$N \equiv$ total number of primary sampling units (plots) in a stratum

$n \equiv$ total number of primary sampling units sampled (plots) in a stratum

$M_i \equiv$ total number of secondary sampling units (lines) in plot i

$m_i \equiv$ total number of secondary sampling units (lines) in sampled in plot i

$y_{ij} \equiv$ mean number of shoots per m^2 within the sample quadrats on the line j in plot i scaled up to the sampled transect width ($\times 92.9$ for mitigation or cultch); ($\times 116.1$ for baskets)

We can calculate the following quantities:

1. $\bar{y}_i = \frac{1}{m_i} \sum_{j=1}^{m_i} y_{ij}$, the average number of shoots per transect in plot i
2. $\hat{y}_i = \frac{M_i}{m_i} \sum_{j=1}^{m_i} y_{ij} = M_i \bar{y}_i$, the total number of shoots in plot i
3. $\hat{\tau} = \frac{N}{n} \sum_{i=1}^n \hat{y}_i$, an unbiased estimator of the total number of shoots in the stratum
4. $\hat{\mu}_1 = \frac{1}{n} \sum_{i=1}^n \hat{y}_i = \frac{\hat{\tau}}{N}$, an unbiased estimator of the population mean number of shoots per plot
5. $s_u^2 = \frac{1}{n-1} \sum_{i=1}^n (\hat{y}_i - \hat{\mu}_1)^2$, the sample variance for between plot total shoots per plot
6. $s_i^2 = \frac{1}{m_i-1} \sum_{j=1}^{m_i} (y_{ij} - \bar{y}_i)^2$, the sample variance for within plot i
7. $\widehat{\text{Var}}(\hat{\tau}) = N(N-n) \frac{s_u^2}{n} + \frac{N}{n} \sum_{i=1}^n M_i (M_i - m_i) \frac{s_i^2}{m_i}$, the estimated variance for the total number of shoots in the stratum
8. $\widehat{\text{Var}}(\hat{\mu}_1) = \frac{\widehat{\text{Var}}(\hat{\tau})}{N^2}$, the estimated variance for the mean number of shoots per plot

The total estimated variance is a function of the observed between plot and within plot variances.

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